Low diversity of helminth parasites in Sardina pilchardus and Engraulis encrasicolus (Clupeidae) from the Bay of Biscay

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Abstract

Parasitological data are increasingly used to provide information on host populations, trophic interactions and free-living biodiversity. Here, parasitic helminths have been researched in *Sardina pilchardus* and *Engraulis encrasicolus* from the Bay of Biscay (North-East Atlantic). In addition, a review of helminths infecting *E. encrasicolus* has been provided. Surprisingly, our field study reveals the occurrence of only three species of Ascaridoidea (*Anisakis simplex* sensu stricto, *Anisakis pegreffi* and *Hysterothylacium aduncum*) in both fish species despite the high diversity of helminth parasites (mostly heteroxenous trophically-transmitted) recorded throughout their distribution range, i.e. 39 and 15 taxa, respectively, for *S. pilchardus* and *E. encrasicolus:* among them, 12 shared taxa. This low diversity of helminth parasites in the Bay of Biscay may be indicative of a low free-living biodiversity. Moreover, similarities in the helminth parasites of *S. pilchardus* and *E. encrasicolus,* recorded both in our field study and in their entire geographical distribution, suggest ecological and feeding similarities between these two planktivorous fish. However, the higher prevalence and mean intensity of Ascaridoidea in *E. encrasicolus* (vs. *S. pilchardus*) in the Bay of Biscay also highlighted differences, which were considered the result of its higher trophic plasticity.

Keywords: small pelagic fish, sardine, anchovy, helminth parasites, Ascaridoidea, low biodiversity, feeding ecology.

Introduction

Small pelagic fish, such as *Sardina pilchardus* and *Engraulis encrasicolus*, contribute up to 50% of the total landing of marine species worldwide and play a considerable role in connecting the lower and upper trophic levels (Fréon *et al.* 2005; Palomera *et al*. 2007; Morello and Arneri 2009 for reviews). First, they are both suspension and raptorial feeders, and their flexible, dual planktivorous diet includes diverse taxa, e.g. crustaceans of various sizes (copepods, cladocerans, euphausiids, amphipods and decapods), appendicularians and fish eggs and larvae, depending on the season and fish size (Bacha and Amara 2009; Morello and Arneri 2009; Petitgas 2010; Atkinson *et al.* 2014; Chouvelon *et al*. 2014, 2015). Second, they are preyed on by many predator species such as planktonic chaetognaths (as larval fish), demersal and medium to large pelagic fish, marine mammals, and seabirds (Morello and Arneri 2009; Petitgas 2010; Certain *et al.* 2011). As a consequence, *S. pilchardus* is the host of a great number of parasites, including 39 helminth taxa, most of which are trophically-transmitted (Marzoug *et al.* 2012 for review). In the same way, parasites of *E. encrasicolus* are probably highly diverse.

Helminth parasites are increasingly used to provide information on host populations, potentially helping manage fish stocks, free-living biodiversity and changes in ecosystem structure and functioning (MacKenzie 2002; Marcogliese 2004 for reviews).

In this study, helminth parasites have been investigated in adults of *S. pilchardus* and *E. encrasicolus* sampled in different sites across the Bay of Biscay (North-East Atlantic). In addition, helminth parasites of *E. encrasicolus* have been reviewed in their entire geographical distribution. The results are discussed in relation to the potential role of helminth parasites as indicators of food webs, free-living biodiversity and ecosystem stress.

Materials and Methods

Study site and samplings

PELGAS (PELagique GAScogne) is an annual pelagic acoustic spring survey that has been led by the French Research Institute for Exploitation of the Sea (IFREMER) since 2000 in the Bay of Biscay. It aims to monitor the abundance of small pelagic fish in order to help conserve fish stocks. In spring 2012, PELGAS provided 49 *S. pilchardus* and 52 *E. encrasicolus* collected by pelagic trawling (for 30 to 45 min) at various locations and depths in the survey area (43°30' to 49°00'N, 1°10' to 6°00'W) (Fig. 1, Table 1). All fish were at an adult stage according to their age determined by otolithometry (Soares *et al.* 2007, ICES 2010) (mean age of 1.76 ± 0.06 years for *S. pilchardus* and 1.92 ± 0.04 years for *E. encrasicolus*).

Parasitological research

All fish were measured (cm) and frozen before the complete examination of helminth parasites, as in previous studies (Gérard *et al.* 2013, 2015). All organs and tissues (skin, muscles, eyes, brain, gills, heart, body cavity, digestive system, gonads and swimbladder) were meticulously dissected under a binocular stereomicroscope. All the helminth parasites found were numbered. The identification of the nematodes (i.e., Ascaridoidea) was firstly based on morphological criteria according to Fagerholm (1991) and Anderson *et al.* (2009), demonstrating the occurrence of two genera, i.e. *Anisakis* and *Hysterothylacium*. Secondly, molecular identification at the species level via DNA sequencing (Cytochrome Oxidase sub-unit 2, cox2) was realised on all *Anisakis* (75) and some *Hysterothylacium* (10) specimens preserved in alcohol (70%). Total DNA of parasites was extracted from all individual nematode tissues using the ChargeSwitch® Forensic DNA Purification Kit (Invitrogen). A 530 bp fragment of the cox2 gene was amplified in collaboration with the

French National Research Agency (ANR) Fish-Parasites project (data not yet published). Automated DNA sequencing was performed by Genoscreen (Lille, France), then sequences were analysed using BioEdit software (Hall, 1999) and compared with nucleotide sequences from GenBank (National Center for Biotechnology Information) with the programme BLAST (Basic Local Alignment Search Tool).

The parasite descriptors used were: prevalence (number of hosts infected with a particular parasite taxon / number of hosts examined) and mean intensity (average intensity of a particular parasite taxon among the infected members of a particular host species) (Bush *et al.* 1997).

Statistical analyses

Statistical analyses were made with the R-Cran project free software (R Development Core Team 2011). Differences were considered statistically significant at $p < 0.05$. Mean values of data are reported as means ± 95% confidence limits (CL).

General linear models (GLMs (McCullagh 1984)) followed by an analysis of variance (ANOVA, Chi-squared test) were set up to check parasitological differences between *S. pilchardus* and *E. encrasicolus* in terms of prevalence (binomial model) and mean intensity (Poisson model) of parasites (Hastie and Pregibon 1992). Then, inter-site comparisons were performed by successively modifying the reference site in the GLMs.

Results

Helminth parasites of S. pilchardus and E. encrasicolus in the Bay of Biscay

All parasites recovered were nematodes belonging to the super family of Ascaridoidea (*Anisakis* and *Hysterothylacium* genera) at the third larval stage in the body cavity or adults in the digestive system. No helminth parasites were detected in the other organs. The

overall prevalence of helminth parasites was 77.6% (CL = 64–88) for *S. pilchardus* (49) and 96.2% (87–100) for *E. encrasicolus* (52), with a mean intensity of, respectively, 11.92 ± 4.21 and 18.76 ± 5.61. According to the DNA sequences (cox2) and their comparison with sequences present in the GenBank nucleotide database, three parasite species were unambiguously identified in both fish species: *Anisakis simplex* sensu stricto (Anisakidae), *Anisakis pegreffi* (Anisakidae) and *Hysterothylacium aduncum* (Raphidascarididae). Among the 466 nematodes found in *S. pilchardus*, 99.4% belonged to *H. aduncum*, 0.4% to *A. simplex* s.s. and 0.2% to *A. pegreffii*; among the 982 nematodes found in *E. encrasicolus*, 92.3% belonged to *H. aduncum*, 5.7% to *A. simplex* s.s. and 1.7% to *A. pegreffii*.

The overall prevalence and mean intensity of Ascaridoidea were significantly higher in *E. encrasicolus* than in *S. pilchardus* ($p = 0.01$ and 2.10^{-15}, respectively). In the site common to the two fish species (Q450), the mean intensity was also significantly higher for *E. encrasicolus* than for *S. pilchardus* ($p = 5.10^{-16}$), but the prevalence was not significantly different (Fig. 1).

Inter-site differences of Ascaridoidea prevalence were not significant for either fish species (Fig. 1). However, significant differences occurred between some sites in the mean intensity of parasites for both *S. pilchardus* and *E. encrasicolus* (Fig. 1).

Checklist of helminth parasites recorded in E. encrasicolus

Only two complete parasitological investigations of *E. encrasicolus* were found in the literature, i.e. Reichenbach-Klinke (1958) on 100 individuals from the western Mediterranean (Italy) and Shukhgalter (2002) on 290 individuals from the north-eastern Atlantic [north-western coast of Africa (Morocco and Sahara)]. In total, 15 helminth taxa were recorded in *E. encrasicolus* (Table 2); among them were the Ascaridoidea species

found in our field study. All the taxa (except monogenean *Pseudoanthocotyle* sp.) were heteroxenous and trophically-transmitted, with at least three hosts in their life cycle, including invertebrates and vertebrates. Twelve of the 15 helminth taxa listed in *E. encrasicolus* were also infecting *S. pilchardus* (Table 2).

Discussion

Similarities in the helminth parasites of *S. pilchardus* and *E. encrasicolus* recorded both in our field study (three species of Ascaridoidea in the Bay of Biscay) and in their entire geographical distribution (12 trophically-transmitted taxa in common among Digenea, Cestoda, Nematoda and Acanthocephala) highlighted feeding similarities. Such similarities have been previously demonstrated between these two small planktivorous pelagics using analyses of stomach contents and stable isotopes despite a greater trophic plasticity for *E. encrasicolus* (Morello and Arneri 2009; Chouvelon *et al*. 2014, 2015 for reviews). In the Bay of Biscay, *S. pilchardus* and *E. encrasicolus* mainly feed with small- to medium-sized copepods (e.g., *Acartia* sp. and *Temora* sp.) (Chouvelon *et al*. 2014, 2015). These copepods probably act as intermediate hosts for the Ascaridoidea recorded here; *S. pilchardus* and *E. encrasicolus* serving as trophic links in both *Anisakis* and *Hysterothylacium* life cycles (Køie 1993; Mattiucci and Nascetti 2008; Kijewska *et al.* 2009). More precisely, these small pelagic fish are paratenic hosts for *A. simplex* s.s. and *A. pegreffi* harbouring third larval stages (L3) in their body cavity (Mattiucci and Nascetti 2008), and both paratenic hosts (L3 in the body cavity) or definitive hosts (adults in the digestive system) for *H. aduncum* (Køie 1993). The higher prevalence and mean intensity of Ascaridoidea in *E. encrasicolus* than in *S. pilchardus* in the Bay of Biscay (overall and in the common site Q450) can be partly explained by the greater trophic plasticity of *E. encrasicolus* foraging on a wider range of prey sizes, compared to the S. *pilchardus* diet,

which is more limited to mesozooplanktonic species and potentially includes phytoplankton (Chouvelon *et al.* 2014, 2015). Inter-site differences in the mean intensity of Ascaridoidea recorded for each fish species may also suggest local variations in the already described feeding strategies and trophic resources used depending on depth and food availability, as shown through analyses of stomach contents and stable isotopes (Morello and Arneri 2009; Chouvelon *et al.* 2014, 2015 for reviews). One can expect that spatial and temporal variations in the resource availability and in the foraging strategy of small pelagic fish induce variations in their patterns of trophically-transmitted parasites.

The low diversity of helminth parasites found in both fish species studied from the Bay of Biscay (three species of Ascaridoidea) is surprising compared to the 39 taxa for *S. pilchardus* and the 15 taxa for *E. encrasicolus* recorded throughout their distribution range (Marzoug *et al.* 2012; Table 2). For comparison, 10 and 8 taxa of helminth parasites were found respectively in *S. pilchardus* and *E. encrasicolus* from the north-eastern Atlantic (north-western coast of Africa) by Shukhgalter (2002). Because the diversity of helminth parasites is positively correlated with the host diversity, it is increasingly considered to reflect the ecosystem's health and the potential degradation of environmental conditions (e.g., Marcogliese 2004, 2005; Hudson *et al*. 2006; Hechinger *et al*. 2007; Thieltges *et al*. 2011). In the Bay of Biscay, long-term investigations have shown that marine ecosystems are increasingly constrained by the effects of anthropogenic activities (e.g., pollution and over-fishing) and oceano-climatic changes (e.g., water warming and increased stratification) that could threaten the biodiversity in both coastal and pelagic systems (Blanchard *et al*. 2006; Hémery *et al*. 2008; Chust *et al*. 2011). Indeed, changes in the fish assemblages linked to interacting fishing and climatic factors result in the increasing abundance of subtropical fish species (Blanchard *et al*. 2006). Overall, boreal species of

vertebrates (fish, seabirds and marine mammals) with affinities for cold temperate waters declined significantly or even totally disappeared, whereas meridional vertebrates with affinities for hotter waters increased in population size (Hémery *et al*. 2008).

Thus, the low diversity of helminth parasites in *S. pilchardus* and *E. encrasicolus* in the Bay of Biscay may be indicative of changes in free-living diversity with a loss of host diversity. Further extensive investigations on both parasite and free-living diversity in the Bay of Biscay are needed to assess the potential use of helminth parasites as indicators of changes in biocenosis and of stressed marine ecosystems.

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References

Anderson, R. C., Chabaud, A. G., and Willmott, S. (2009). 'Keys to the nematode parasites of vertebrates.' (CABI Head Office: UK.)

Atkinson, A., Hill, S. L., Barange, M., Pakhomov, E. A., Raubenheimer, D., Schmidt, K., Simpson, S. J., and Reiss, C. (2014). Sardine cycles, krill declines, and locust plagues: revisiting 'wasp-waist' food webs. *Trends in Ecology & Evolution* **29**, 309-316.

Bacha, M., and Amara, R. (2009). Spatial, temporal and ontogenetic variation in diet of anchovy (*Engraulis encrasicolus*) on the Algerian coast (SW Mediterranean). *Estuarine, Coastal and Shelf Science* **85**, 257–264.

Blanchard, F., Thébaud, O., Guyader, O., Lorance, P., Boucher, J., and Chevaillier, P. (2006). Effets de la pêche et du réchauffement climatique sur la coexistence

spatiale des espèces de poissons du golfe de Gascogne. Conséquences pour les pêcheries. IFREMER, Programme de recherche « Biodiversité et Changement Global », http://archimer.ifremer.fr/doc/00000/6347/.

Bush, A. O., Lafferty, K. D., Lotz, J. M., and Shostak, A. W. (1997). Parasitology meets ecology on its terms: Margolis et al revisited. *Journal of Parasitology* **84**, 575–583.

Certain, G., Masse, J., Van Canneyt, O., Petitgas, P., Doremus, G., Santos, M. B., and Ridoux, V. (2011). Investigating the coupling between small pelagic fish and marine top predators using data collected from ecosystem-based surveys. *Marine Ecology Progress Series* **422**, 23-39.

Chouvelon, T., Chappuis, A., Bustamante, P., Lefebvre, S., Mornet, F., Guillou, G., Violamer, L., and Dupuy, C. (2014). Trophic ecology of European sardine *Sardina pilchardus* and European anchovy *Engraulis encrasicolus* in the Bay of Biscay (north-east Atlantic) inferred from δ13C and δ15N values of fish and identified mesozooplanktonic organisms. *Journal of Sea Research* **85,** 277–291.

Chouvelon, T., Violamer, L., Dessier, A., Bustamante, P., Mornet, F., Pignon-Mussaud, C., and Dupuy, C. (2015). Small pelagic fish feeding patterns in relation to food resource variability: an isotopic investigation for *Sardina pilchardus* and *Engraulis encrasicolus* from the Bay of Biscay (north-east Atlantic). *Marine Biology* **162**, 15-37. doi:10.1007/s00227-014-2577-5.

Chust, G., Borja, A., Caballero, A., Irigoien, X., Sáenz, J., Moncho, R., Marcos, M., Liria, P., Hidalgo, J., Valle, M., and Valencia, V. (2011). Climate change impacts on coastal and pelagic environments in the southeastern Bay of Biscay. *Climate Research* **48**, 307–332. doi:10.3354/cr00914.

Costa, G., Melo-Moreira, E., and Pinheiro de Carvalho, M. A. A. (2012). Helminth parasites of the oceanic horse mackerel *Trachurus picturatus* Bowdich 1825 (Pisces: Carangidae) from Madeira Island, Atlantic Ocean, Portugal. *Journal of Helminthology* **86**, 368–372.

Dimitrov, G. I., Bray, R. A., and Gibson, D. I. (1999). A redescription of *Pseudobacciger harengulae* (Yamaguti, 1938) (*Digenea: Faustulidae*) from *Sprattus sprattus phalericus* (Risso) and *Engraulis encrasicholus ponticus* Alexandrov off the Bulgarian Black Sea coast, with a review of the genus *Pseudobacciger*Nahhas & Cable, 1964. *Systematic parasitology* **43**, 133–146.

Fagerholm, H.-P. (1991). Systematic implications of male caudal morphology in ascaridoid nematode parasites. *Systematic Parasitology* **19**, 215-228.

Fréon, P., Cury, P., Shannon, L., and Roy, C. (2005). Sustainable exploitation of small pelagic fish stocks challenged by environmental and ecosystem changes: a review. *Bulletin of Marine Science* **76**, 385–462.

Gérard, C., Amilhat, E., Trancart, T., Faliex, E., Virag, L., Feunteun, E., and Acou, A. (2013). Influence of introduced vs native parasites on the body condition of migrant silver eels. *Parasite* **20**(38), doi: 10.1051/parasite/2013040.

Gérard, C., Verrez-Bagnis, V., Jérôme, M., and Lasne, E. (2015). *Petromyzon marinus* (Petromyzontidae), an unusual host for helminth parasites in Western Europe. *Diseases of Aquatic Organisms*, doi: 10.3354/dao02842.

Gutiérrez-Galindo, J. F., Osanz-Mur, A. C., and Mora-Ventura, M. T. (2010). Occurrence and infection dynamics of anisakid larvae in *Scomber scombrus*, *Trachurus trachurus*, *Sardina pilchardus*, and *Engraulis encrasicolus* from Tarragona (NE Spain). *Food Control* **21**, 1550–1555. doi:10.1016/j.foodcont.2010.03.019.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* **41**, 95-98.

Hastie, T. J., and Pregibon, D., (1992). 'Generalized linear models.' (Eds J. M. Chambers and T. J. Hastie, Wadsworth & Brooks/Cole.)

Hechinger, R. F., Lafferty, K. D., Huspeni, T. C., Brooks, A. J., and Kuris, A. M. (2007). Can parasites indicate free-living diversity? Relationships between the species richness and abundance of larval trematodes with that of local benthos and fishes. *Oecologia* **151**, 82-92.

Hémery, G., D'Amico, F., Castege, I., Dupont, B., D'Elbée, J., Lalanne, Y., and Mouches, C. (2008). Detecting the impact of oceano-climatic changes on marine ecosystems using a multivariate index: The case of the Bay of Biscay (North Atlantic-European Ocean). *Global Change Biology* **14**, 27–38.

Hudson, P. J., Dobson, A. P., and Lafferty, K. D. (2006). Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology & Evolution* **21**, 381–385. doi:10.1016/j.tree.2006.04.007.

Kijewska, A., Dzido, J., Shukhgalter, O., and Rokicki, J. (2009). Anisakid Parasites of Fishes Caught on the African Shelf. *Journal of Parasitology* **95**, 639–645.

Køie, M. (1993). Aspects of the life-cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). Canadian *Journal of Zoology* **71**, 1289-1296.

Kostadinova, A., Gibson, D. I., Balbuena, J. A., Power, A. M., Montero, F. E., Aydogdu, A., and Raga, J. A. (2004). Redescriptions of *Aphanurus stossichii* (Monticelli, 1891) and *A. virgula* Looss, 1907 (Digenea: Hemiuridae). *Systematic Parasitology* **58**, 175–184.

MacKenzie, K. (2002). Parasites as biological tags in population studies of marine organisms: an update. *Parasitology* **124**, S153–S163.

Marcogliese, D. J. (2004). Parasites: Small Players with Crucial Roles in the Ecological Theater. *EcoHealth* **1**, 151–164. doi:10.1007/s10393-004-0028-3.

Marcogliese, D. J. (2005). Parasites of the superorganism: Are they indicators of ecosystem health? *International Journal for Parasitology* **35**, 705–716. doi:10.1016/j.ijpara.2005.01.015.

Marzoug, D., Boutiba, Z., Gibson, D. I., Pérez-del-Olmo, A., and Kostadinova, A. (2012). Descriptions of digeneans from *Sardina pilchardus* (Walbaum) (Clupeidae) off the Algerian coast of the western Mediterranean, with a complete list of its helminth parasites. *Systematic Parasitology* **81**, 169–186. doi:10.1007/s11230-011-9335-6.

Mattiucci, S., and Nascetti, G. (2008). Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. *Advances in Parasitology* **66**, 47–148.

McCullagh, P. (1984). Generalized linear models. *European Journal of Operational Research* **16**, 285–292. doi:10.1016/0377-2217(84)90282-0.

Morello E.B., and Arneri, E. (2009). Anchovy and sardine in the Adriatic Sea - An ecological review. *Oceanography and Marine Biology: An Annual Review* **47**, 209-256.

Palomera, I., Olivar, M. P., Salat, J., Sabatés, A., Coll, M., García A., and Morales-Nin, B. (2007). Small pelagic fish in the NW Mediterranean Sea: an ecological review. *Progress in Oceanography* **7**, 377–396.

ICES (2010). Report of the workshop on age reading of European anchovy (WKARA). Sicily, Italy. ICES Cooperative Research Report No. 43.

Petitgas, P. (2010). Life cycle spatial patterns of small pelagic fish in the Northeast Atlantic. ICES Cooperative Research Report No. 306, Denmark.

Piras, M.C., Tedde, T., Garippa, G., Virgilio, S., Sanna, D., Farjallah, S., and Merella, P. (2014). Molecular and epidemiological data on *Anisakis* spp. (Nematoda:

Anisakidae) in commercial fish caught off northern Sardinia (western Mediterranean Sea). *Veterinary Parasitology* **203**, 237–240.

Poulin, R. and Leung, T. L. F. (2011). Body size, trophic level, and the use of fish as transmission routes by parasites. *Oecologia* **166**, 731–738.

Soares, E., Silva, A., and Morais, A. (2007). Workshop on sardine otolith age reading and biology. Cient. Téc. Inst. Invest. Pescas Mar Série digital (http://ipimar-iniap.ipimar.pt). No. 42, Lisboa, Portugal.

Thieltges, D. W., Amundsen, P.-A., Hechinger, R. F., Johnson, P. T. J., Lafferty, K. D., Mouritsen, K. N., Preston, D. L., Reise, K., Zander, C. D., and Poulin, R. (2013). Parasites as prey in aquatic food webs: implications for predator infection and parasite transmission. *Oikos* **122**, 1473–1482. doi:10.1111/j.1600-0706.2013.00243.x

Table 1: Geographical position (latitude and longitude in decimal degrees) and water depth (m) of trawls, number (N) and mean total length (TL ± CL, cm) of *Sardina pilchardus* and *Engraulis encrasicolus* sampled in spring 2012 across the Bay of Biscay.

	Trawls	Latitude	Longitude	Depth (m)	N	TL ± CL (cm)
S. pilchardus (N = 49)	Q0380	43.87	-1.74	119.2	9	20.62 ± 0.61
	Q0402	44.47	-1.68	108.7	10	21.05 ± 0.48
	Q0403	44.67	-1.40	45.5	10	21.65 ± 0.56
	Q0434	45.66	-1.41	23.3	10	21.25 ± 0.82
	Q0450	45.57	-3.30	157.2	10	21.40 ± 0.53
E. encrasicolus (N = 52)	Q0379	43.86	-1.99	140.5	10	16.40 ±0.82
	Q0385	44.07	-1.67	112.7	10	15.76 ± 0.49
	Q0401	44.47	-1.89	129.6	10	18.22 ± 1.99
	Q0450	45.57	-3.30	157.2	10	17.05 ± 0.27
	Q0455	46.13	-2.47	79.3	12	12.27 ± 0.67

Table 2: Checklist of the helminth parasite taxa (15) recorded in *Engraulis encrasicolus*.

BS: Baltic Sea, NEA: North East Atlantic, WM: West Mediterranean.

*taxa (12) also recorded in *Sardina pilchardus* (Marzoug *et al*. 2012).

Parasites	Area	References
MONOGENEA		
Fam. Mazocraeidae		
Pseudoanthocotyle sp.	NEA	Shukhgalter 2002
DIGENEA		
Fam. Acanthocolpidae		
Stephanostomum sp. (metacercariae)*	NEA	Shukhgalter 2002
Fam. Bucephalidae		
Bucephalus sp. (metacercariae)*	WM	Reichenbach-Klinke 1958
Fam. Faustulidae		
*Bacciger bacciger**	NEA	Shukhgalter 2002
*Pseudobacciger harengulae**	BS	Dimitrov et *al*. 1999
Fam. Hemiuridae		
*Aphanurus stossichii**	NEA, WM	Reichenbach-Klinke 1958; Shukhgalter 2002
*Aphanurus virgula**	BS	Kostadinova et *al*. 2004
Fam. Lecithasteridae		
Aponurus lagunculus	WM	Reichenbach-Klinke 1958
*Lecithaster confusus**	NEA	Shukhgalter 2002
CESTODA		
Fam. Lacistorhynchidae		
Lacistorhynchus sp. (plerocercoid)	NEA	Shukhgalter 2002
Fam. Tetraphyllidae		
Scolex pleuronectis (plerocercoid)*	NEA, WM	Reichenbach-Klinke 1958; Shukhgalter 2002
NEMATODA		
Fam. Anisakidae		
Anisakis simplex s.l. (larvae) *	NEA	Shukhgalter 2002
Anisakis pegreffi (larvae)*	WM	Piras et *al*. 2014
Fam. Raphidascarididae		
*Hysterothylacium aduncum**	NEA, WM	Reichenbach-Klinke 1958; Shukhgalter 2002
ACANTHOCEPHALA		
Fam. Rhadinorhynchidae		
Rhadinorhynchus sp.*	NEA	Shukhgalter 2002

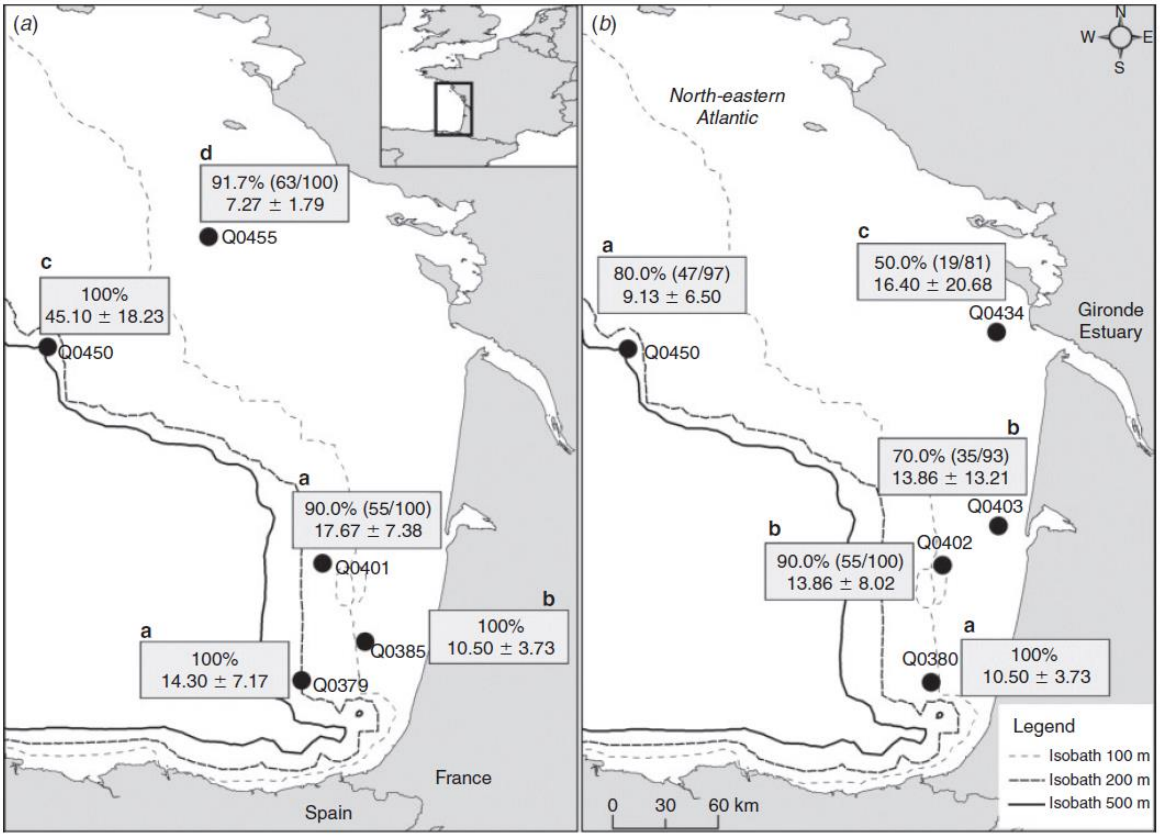

Figure 1: Prevalence % (± CL) and mean intensity (± CL) of Ascaridoidea in *Engraulis encrasicolus* (A) and *Sardina pilchardus* (B) sampled in spring 2012 across the Bay of Biscay. Inter-site differences of prevalence for each fish species were not significant ($p > 0.05$). Significant differences between mean intensities according to the site for each fish species are indicated by letters (a, b, c, d).